

SUPPLEMENTARY INFORMATION

Title: Fructose reprograms glutamine-dependent oxidative metabolism to support LPS-induced inflammation

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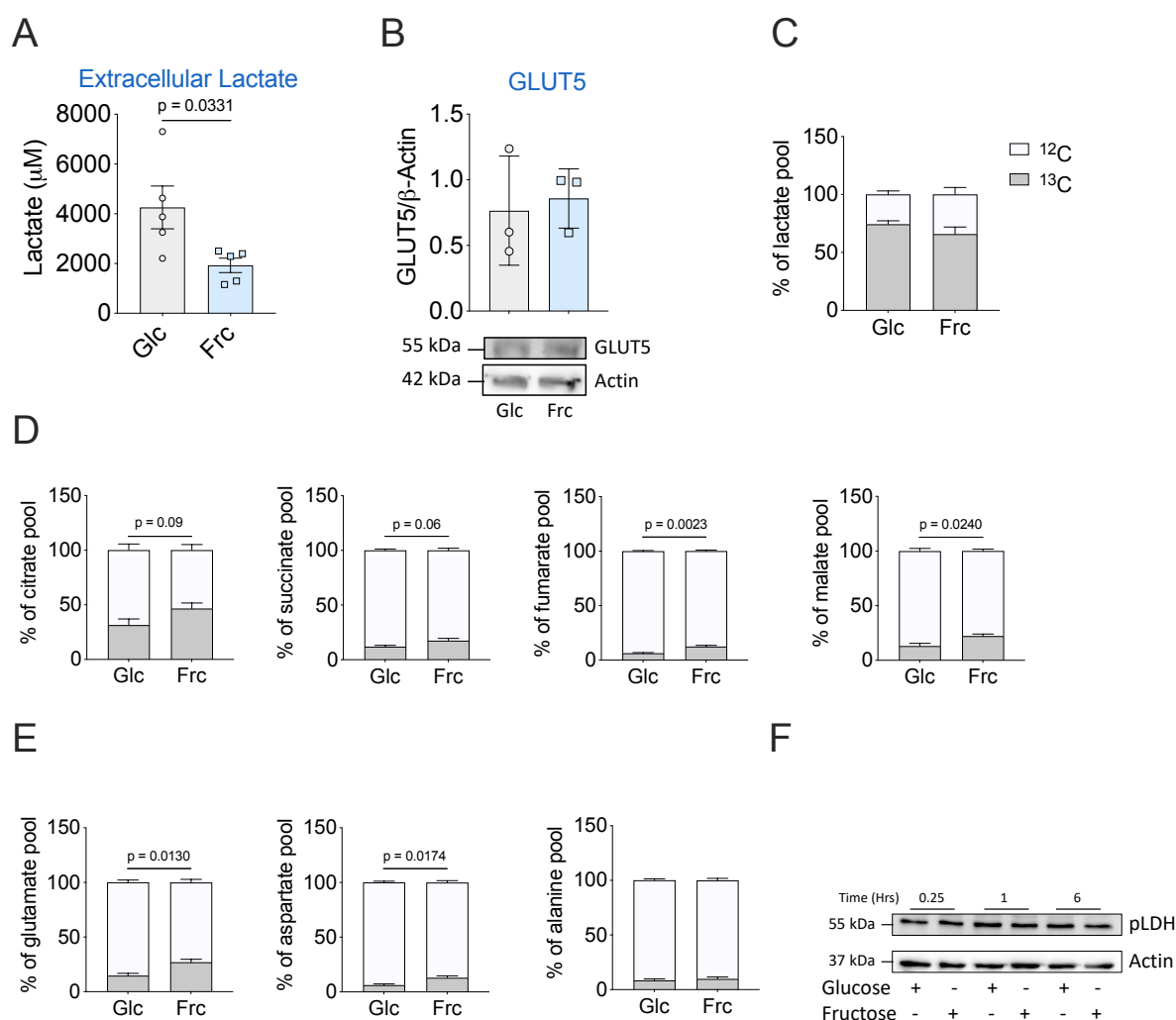
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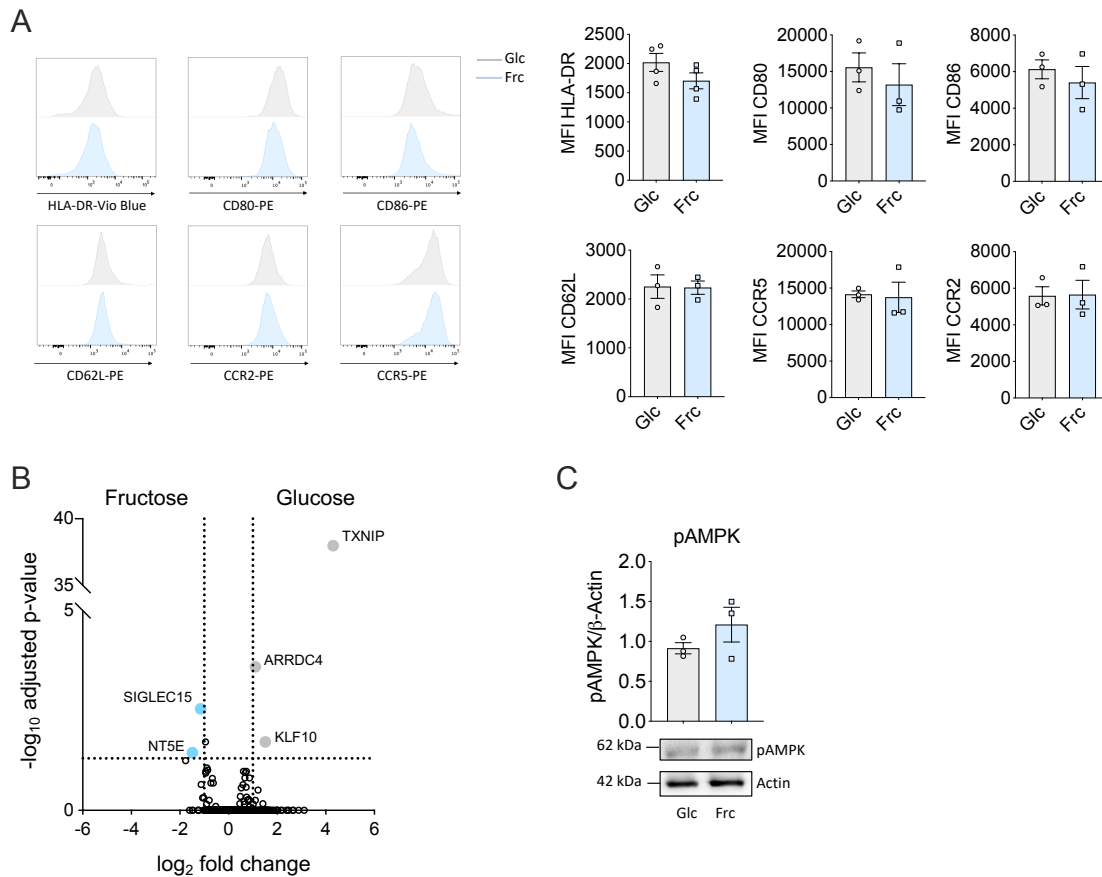
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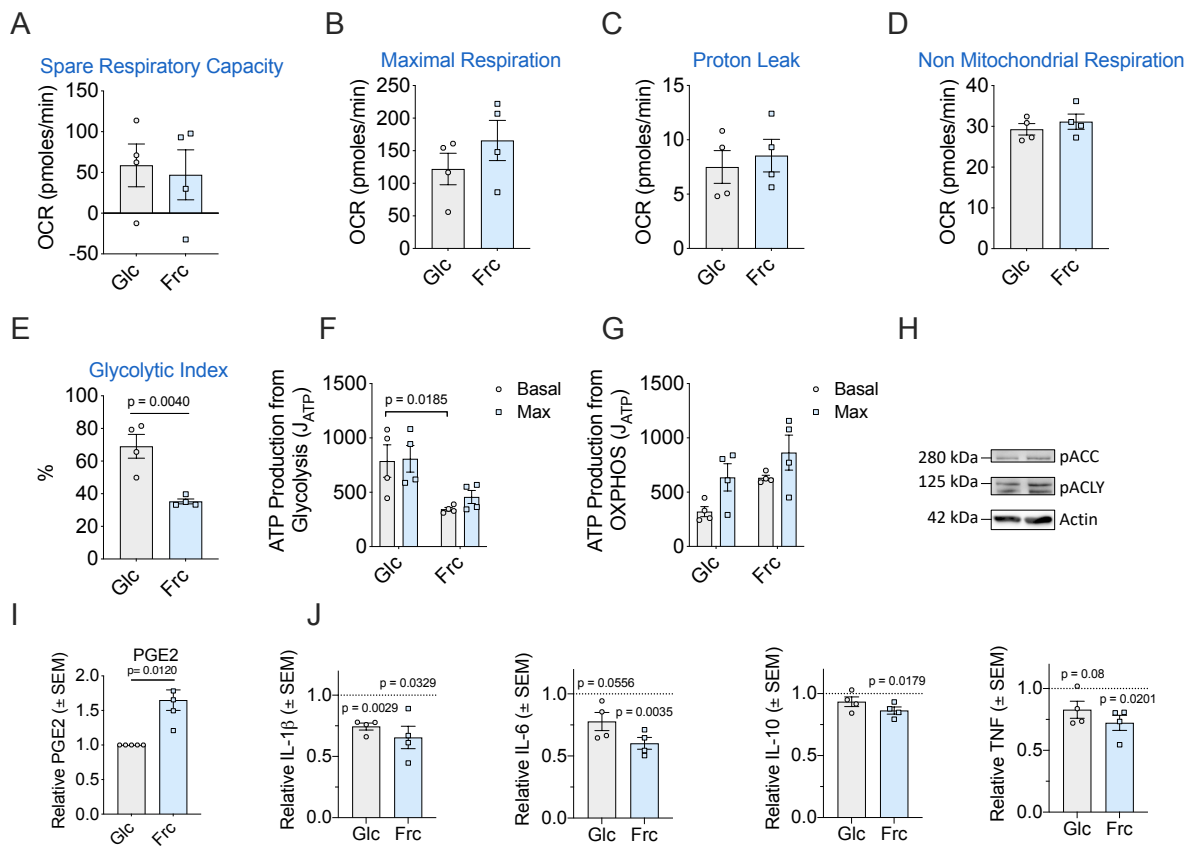
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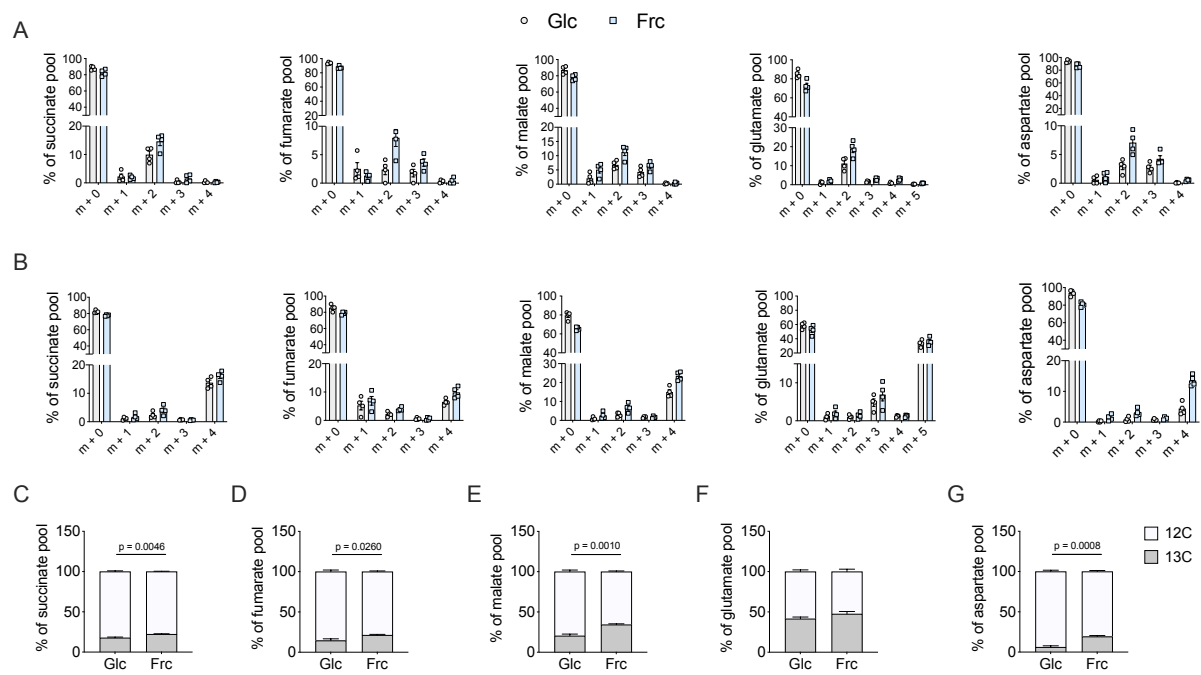
Supplementary Figure 1. Incorporation of fructose-derived carbons into lactate, TCA cycle and amino acid intermediates. (a) Extracellular levels of lactate produced in human monocytes ($1.0 \times 10^6/\text{mL}$) cultured with glucose or fructose for 24 hours in the presence of LPS (10 ng/mL). (b) Immunoblot of glucose transporter 5 (GLUT5) in monocytes cultured with glucose or fructose for 24 hours in the presence of LPS (10 ng/mL) with actin used as the housekeeping control. Percentage of pool of labelled $^{13}\text{C}_6$ -glucose or $^{13}\text{C}_6$ -fructose into (c) lactate, (d) TCA cycle intermediates, citrate, succinate, fumarate and malate or (e) amino acids glutamate, aspartate, alanine and proline. Statistical analysis performed on ^{13}C data only (f) Immunoblot of pLDH in monocytes cultured in glucose or fructose (11.1 mM) and LPS (10 ng/mL) for 0.25, 1 and 6 hours. Statistical significance was assessed using an unpaired, two-tailed t-test (a-e). Data representative of five (a), three (b), four (c-e) or two (f) independent experiments. Data are expressed as mean \pm SEM. Source data are provided as a Source Datafile.



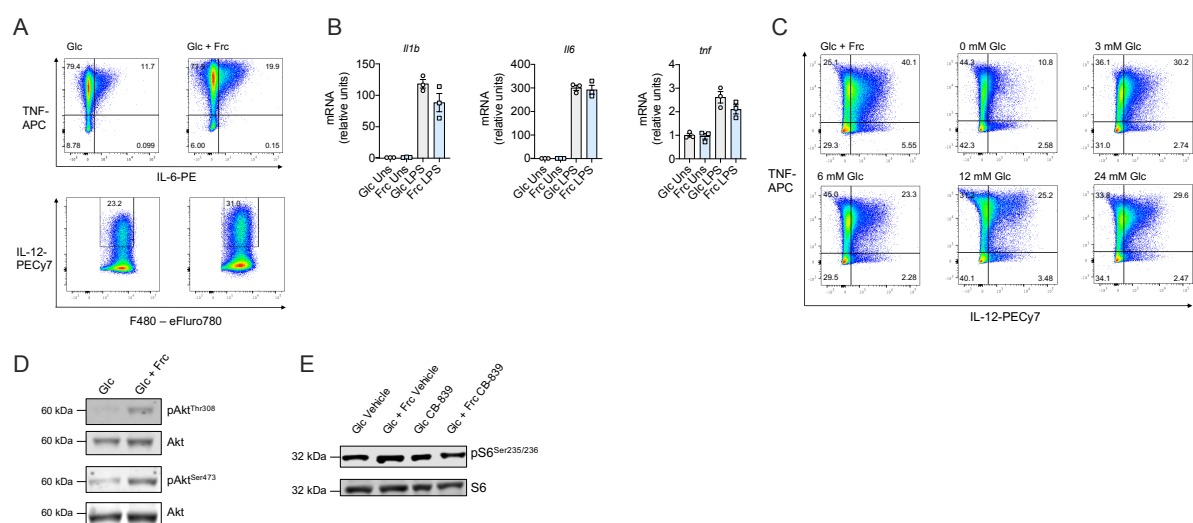
Supplementary Figure 2. Phenotypic surface markers and cytokine transcript levels are not affected by fructose culture. (a) Flow cytometry of phenotypic surface markers (HLA-DR, CD80, CD86, CD62L, CCR5 and CCR2) in monocytes cultured in glucose or fructose (11.1 mM) and LPS (10 ng/mL) for 24 hours. (b) Volcano plot showing the differentially regulated genes ($\log_2FC > \pm 1$, adjusted $P < 0.05$; labelled) when comparing LPS-stimulated (10 ng/mL) monocytes treated with either glucose or fructose (11.1 mM). Genes significantly upregulated by glucose are coloured grey ($n=3$), and genes significantly upregulated by fructose are coloured blue ($n=3$). (c) Immunoblot of phospho-AMPK in monocytes cultured with glucose or fructose for 24 hours in the presence of LPS (10 ng/mL) with actin used as the housekeeping control. Statistical significance was assessed using an unpaired, two tailed t-test (a, c). Data are representative of three (a) with the exception of HLA-DR which is representative of four, or three (b-c) independent experiments and expressed as mean \pm SEM. Source data are provided as a Source Datafile.



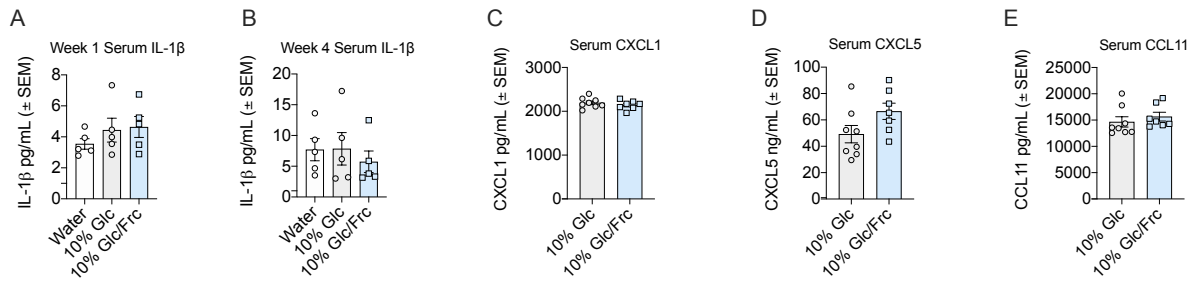
Supplementary Figure 3. Additional metabolic parameters. Oxidative parameters (a) spare respiratory capacity, (b) maximal respiration, (c) proton leak and (d) non mitochondrial respiration of monocytes cultured with glucose or fructose (11.1 mM) and stimulated with LPS (10 ng/mL) for 24 hours. (e) Percentage glycolytic index and ATP production rate (J_{ATP}) from (f) glycolysis or (g) oxidative phosphorylation. Monocytes cultured as above and (h) phosphorylation status of acetyl-CoA carboxylase (ACC) and ATP citrate lyase (ACLY) assessed by immunoblot with actin used as housekeeping control. (i) Extracellular release of prostaglandin E2 (PGE2) of monocytes cultured as previous. (j) Cytokine release (% of control) of IL-1 β , IL-6, IL-10 and TNF (dotted line indicates control) of monocytes cultured in the presence or absence of ACLY inhibitor, BMS303141 (10 μ M). Statistical significance was assessed using an unpaired, two-tailed t-test (a-e, i), two-way ANOVA with Sidak's multiple comparison test (f-g) or a one sample t test (j). Data representative of four (a-g, j), two (h) or five (i) independent experiments. Data are expressed as mean \pm SEM. Source data are provided as a Source Datafile.



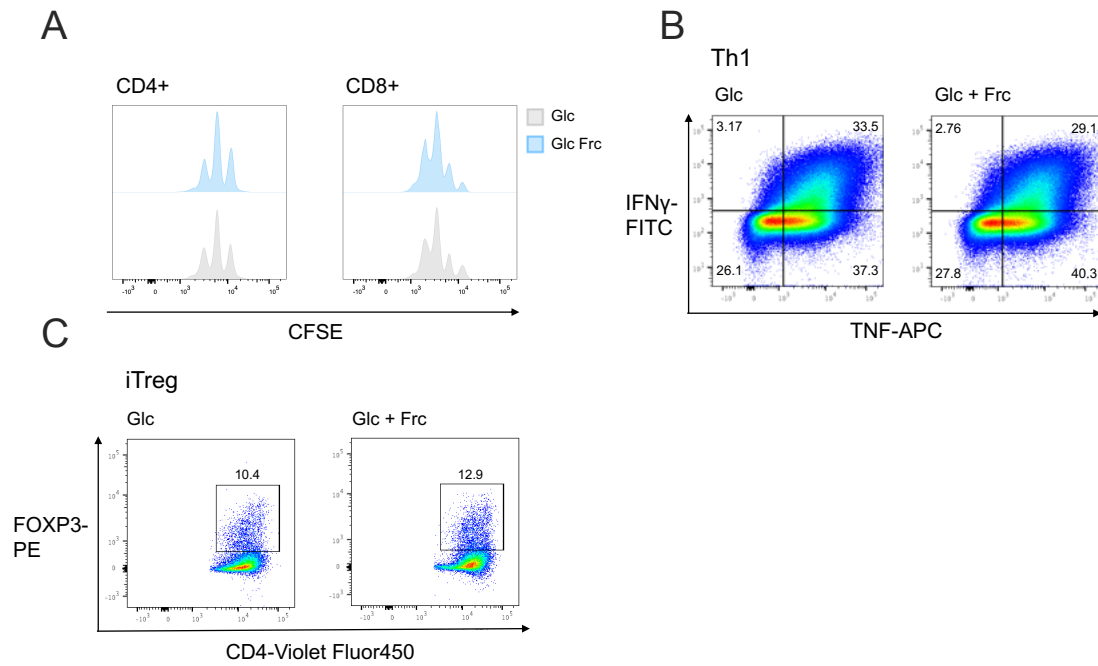
Supplementary Figure 4. Mass isotopologue distributions and incorporation of ^{13}C -glutamine into TCA cycle and amino acid metabolites. (a) Stable isotope tracing analysis (SITA) of 24-hour LPS-stimulated (10 ng/mL) monocytes cultured with uniformly labelled ^{13}C -glucose or ^{13}C -fructose into TCA cycle intermediates (succinate, fumarate and malate) and amino acids (glutamate and aspartate) with the mass isotopologue distribution (MID) represented as a % pool. (b) SITA of uniformly labelled ^{13}C -glutamine in the presence of ^{12}C -glucose or ^{12}C -fructose of 24-hour LPS-stimulated (10 ng/mL) monocytes with % MID shown. Stable isotope tracing of uniformly labelled ^{13}C -glutamine in the presence of ^{12}C -glucose or ^{12}C -fructose of 24-hour LPS-stimulated (10 ng/mL) monocytes. Percentage of pool of metabolites (c) succinate, (d) fumarate, (e) malate, (f) glutamate and (g) aspartate. Statistical significance for was assessed using an unpaired, two-tailed t test on the ^{13}C data (c-g). Data are representative of four (a-c) independent experiments. Data are expressed as mean \pm SEM. Source data are provided as a Source Datafile.



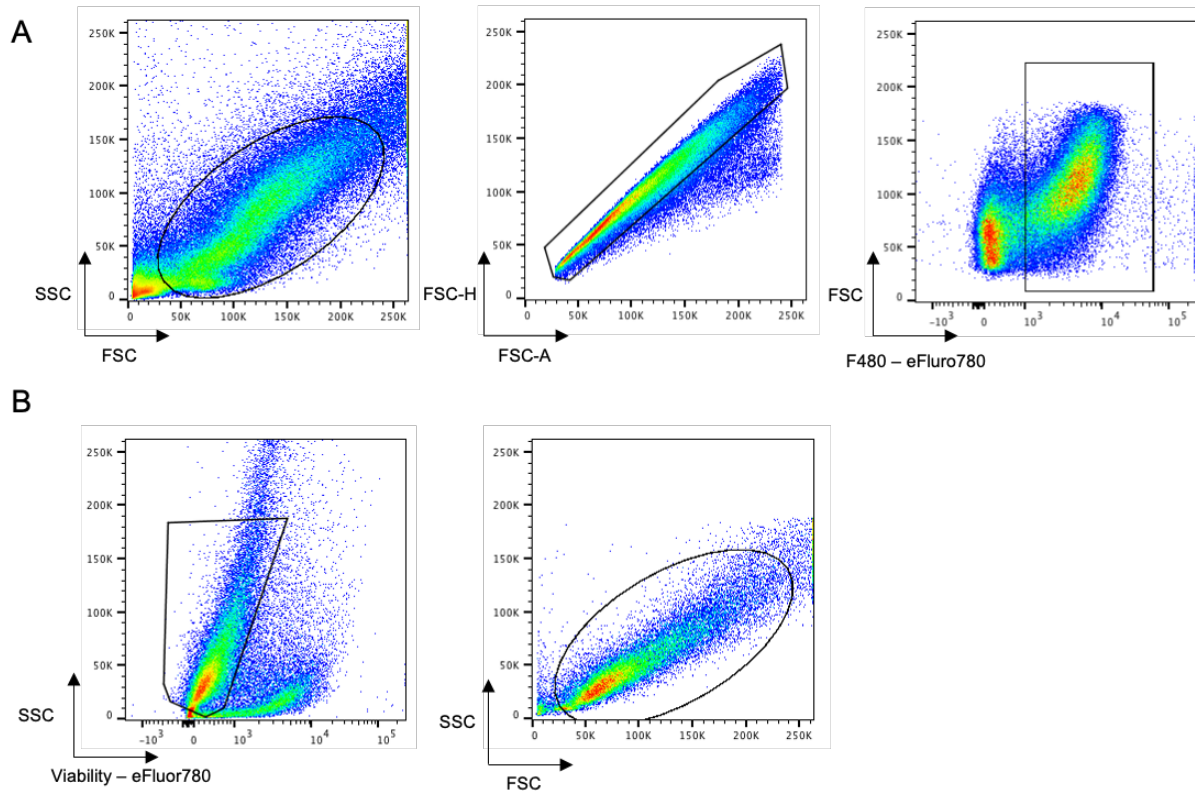
Supplementary Figure 5. Fructose in the presence of glucose enhances inflammation in macrophages (a) Flow cytometry analysis of LPS-stimulated (1 ng/ml) mouse macrophages cultured with glucose (24 mM) or glucose/fructose (both 12 mM) for intracellular cytokine production of IL-6 and IL-12. (b) qPCR for *Il1b*, *Il6* and *Tnf* of mouse macrophages unstimulated or stimulated with LPS (1 ng/mL) for 18 hours cultured in either glucose (24 mM) or glucose/fructose (both 12 mM). (c) Intracellular cytokine staining (ICS) for TNF and IL12 in mouse macrophages cultured with varying glucose concentrations (24, 12, 6, 3 and 0 mM) stimulated with LPS (1 ng/ml) for 5 hours. (d) Immunoblot of phospho-Akt^{Thr308} and phospho-Akt^{Ser473} in macrophages cultured with glucose (24 mM) or glucose/fructose (both 12 mM) for 18 hours in the presence of LPS (1 ng/ml) with total Akt used as the housekeeping control. (e) Immunoblot analysis of p-S6^{Ser235/236} in lysates from macrophages stimulated with 1 ng/ml of LPS and either cultured with glucose (24 mM) or glucose/fructose (both 12 mM) in the presence or absence of the glutaminase inhibitor, CB-839 (1 μ M). Data are representative of four (a, c) or three (b, d-e) independent experiments. Data are expressed as mean \pm SEM (b). Source data are provided as a Source Datafile.



Supplementary Figure 6. Serum cytokine and chemokine production in unchallenged and LPS-challenged mice on sugar water. Serum IL-1 β levels of control mice given *ad libitum* 10% glucose or 10% glucose-fructose water for (a) 1 week and (b) 4 weeks. Serum chemokines (c) CXCL1, (d) CXCL5 and (e) CCL11 production of mice provided 10% glucose or 10% glucose-fructose water for 2 weeks followed by intraperitoneal injection of 0.1 mg/kg LPS for 3 hours. Statistical significance was assessed using a one-way ANOVA with Tukey's multiple comparison test (a-b) or unpaired t-test (c-e). Data are representative of 5 (a-b) or 7-8 (c-e) independent experiments. Data are expressed as mean \pm SEM. Source data are provided as a Source Datafile.



Supplementary Figure 7. Fructose in the presence of glucose does not affect T cell proliferation or CD4 T-cell polarization (a) Representative flow cytometry plots of CFSE-stained CD4⁺ or CD8⁺ T-cells cultured with glucose (24 mM) or glucose-fructose (both 12 mM) and activated with anti-CD3 (5 μ g/ml) and anti-CD28 (2 μ g/ml) for 72 hours. Dead cells were excluded using Fixable Viability dye eFluor780 (b) (c) Data are representative of 3 (a-c) independent experiments.



Supplementary Figure 8. Example gating strategies for macrophages. Gating strategy used to analyse mouse macrophages using **(a)** F480 eFluro780 (related to Figure 7A) or **(b)** dead cell exclusion using viability dye eFluor780 (related to Figure 7G).

Figure 3G



Figure 4J

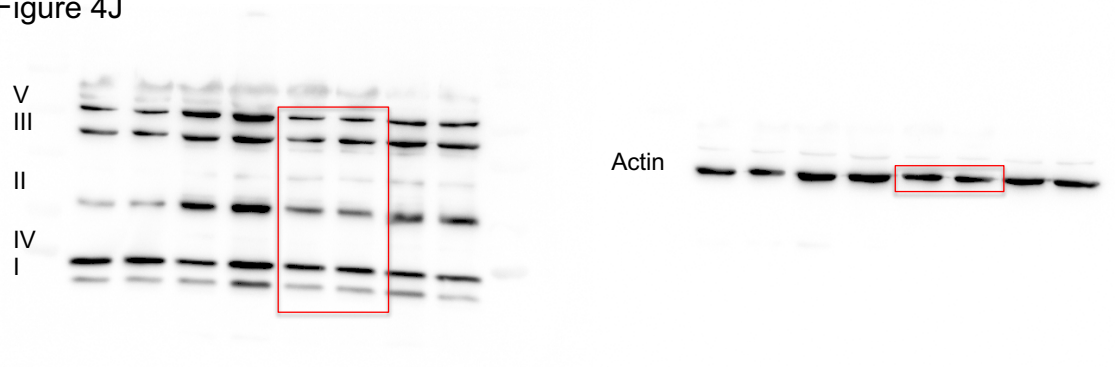
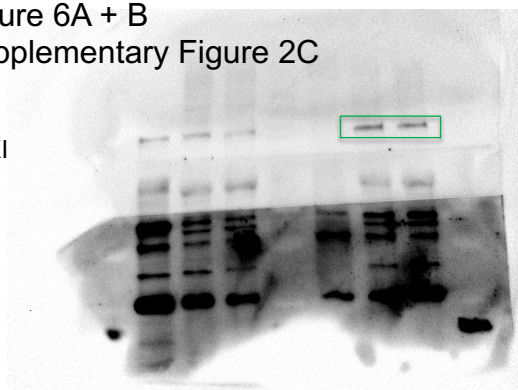


Figure 6A + B
Supplementary Figure 2C

HKI



HKII



Actins - imaged together
Gel 1



Gel 2



Gel 3

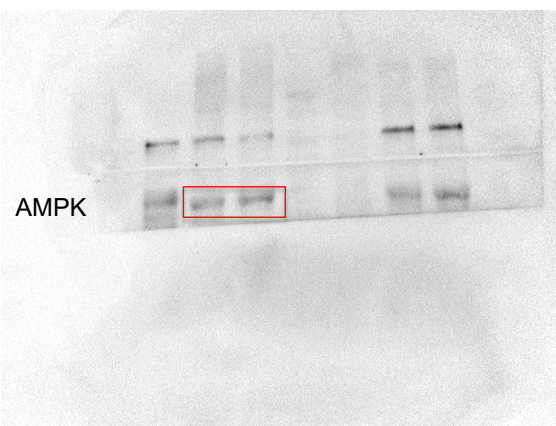
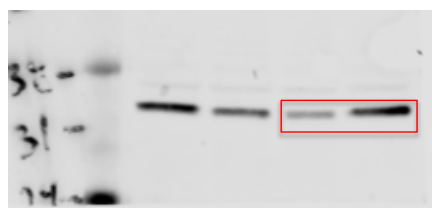
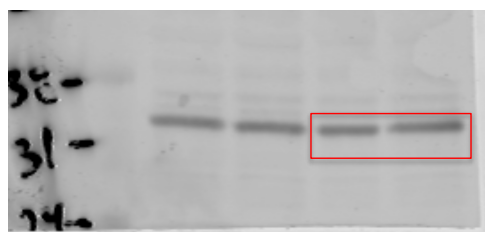


Figure 7E

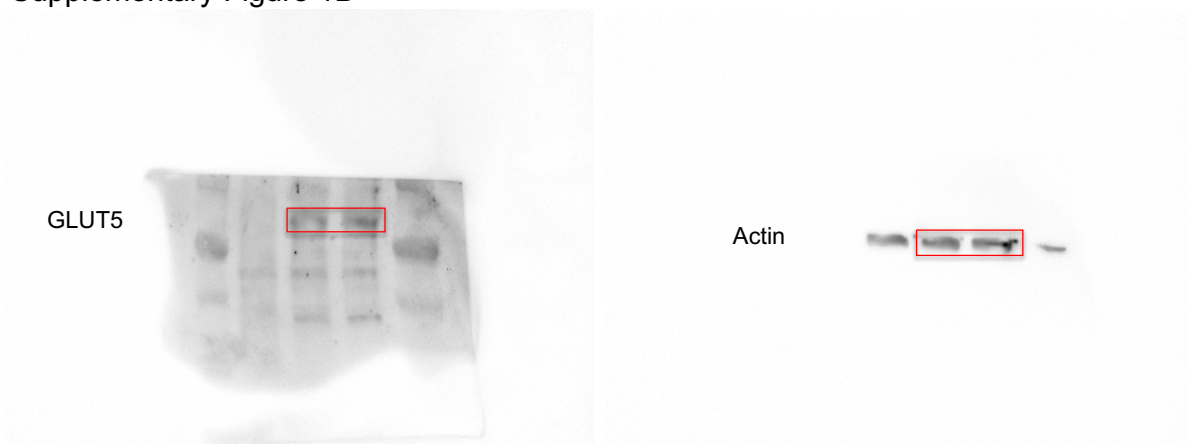
pS6^{Ser235/236}



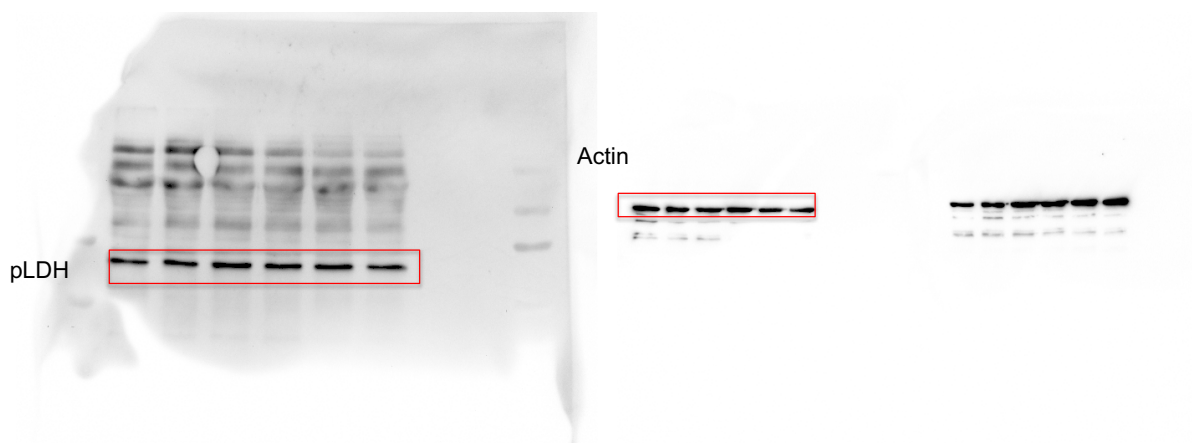
S6



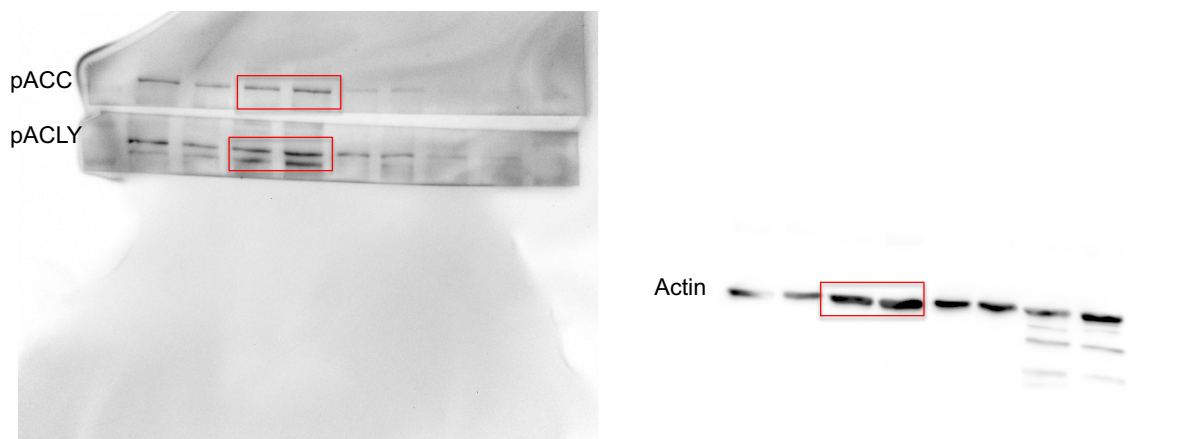
Supplementary Figure 1B



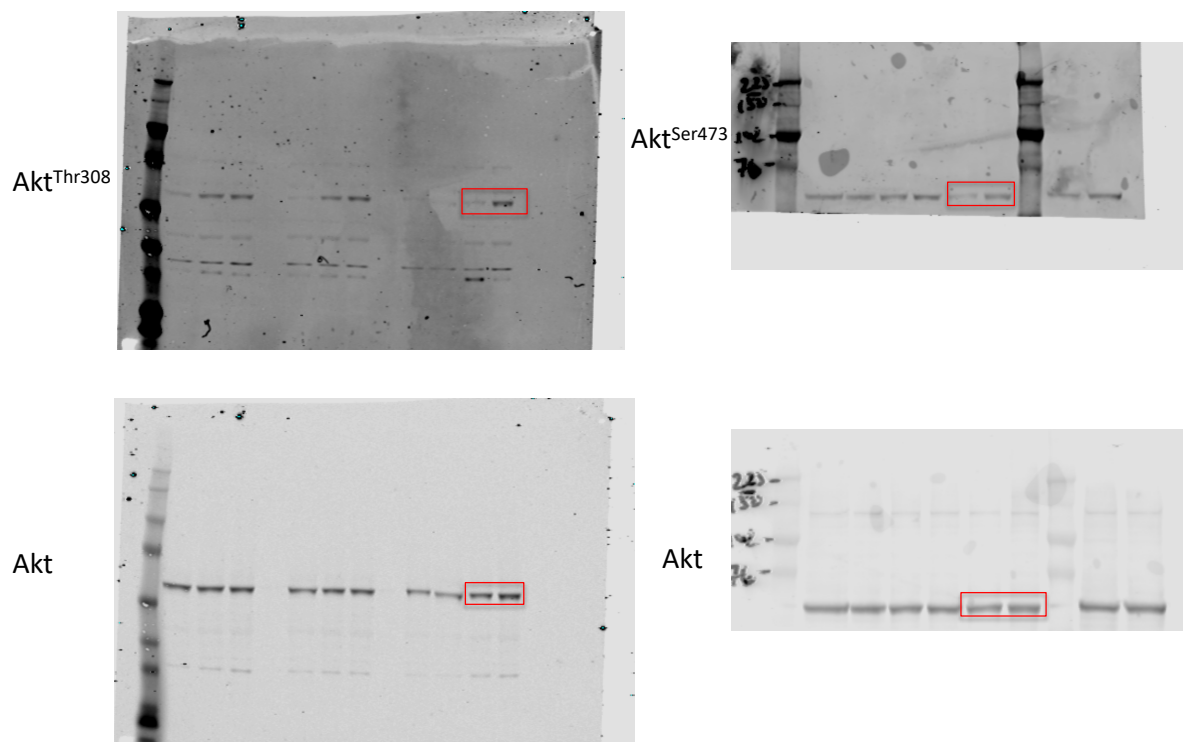
Supplementary Figure 1F



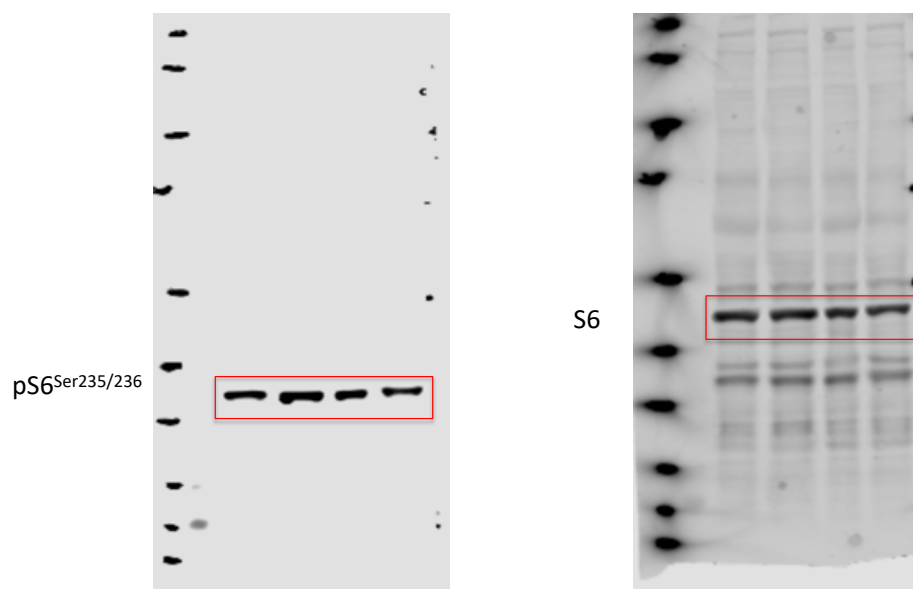
Supplementary Figure 3H



Supplementary Figure 5D



Supplementary Figure 5E



Supplementary Figure 9. Original uncropped immunoblots for main figures and supplementary figures.

Supplementary Table 1 - Primer sequences for qPCR

Gene	Forward primer	Reverse primer
<i>Il6</i>	TCCTACCCCAATTTCCAATGCTC	TTGGATGGTCTTGGTCCTTAGCC
<i>Tnf</i>	CCCCAAAGGGATGAGAAGTT	CTCCTCCACTTGGTGGTTTG
<i>Il1b</i>	GCAACTGTTCTGAACTCAACT	TCTTTTGGGGTCCGTCAACT
<i>cyc</i>	ATGGTCAACCCCACCGTGT	TTTCTGCTGTCTTTGGAACCTTGTC